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Adv. Exp. Med. Biol. (1998), 454(Oxygen Transport to Tissue XX), 311-317

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Thanks,

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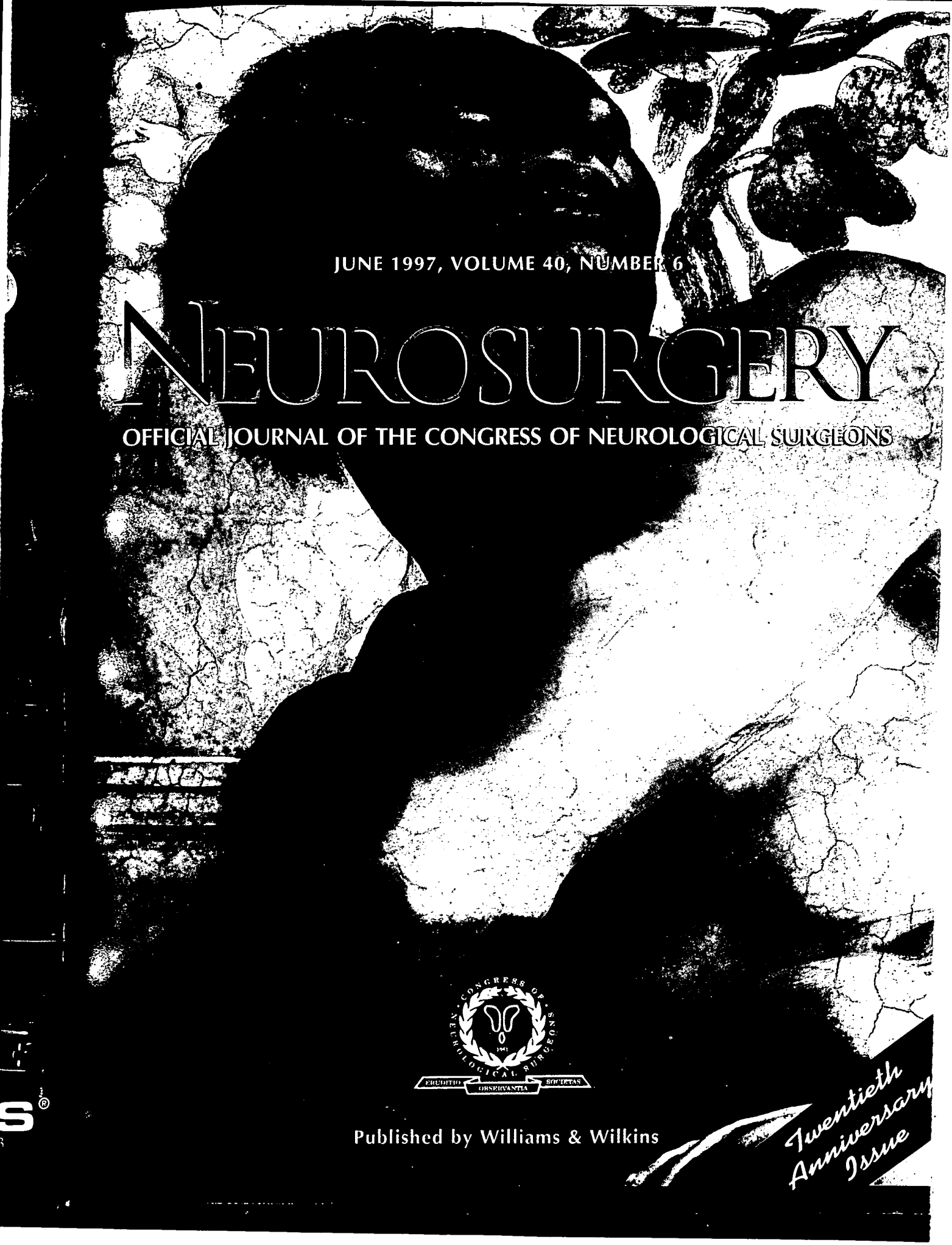

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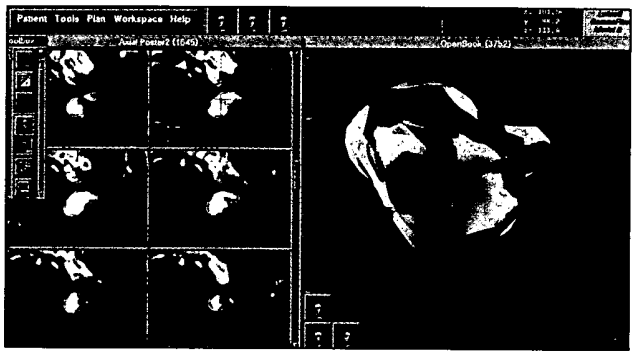

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Brain Edema in Meningiomas Is Associated with Increased Vascular Endothelial Growth Factor Expression

Corey K. Goldman, M.D., Suman Bharara, M.S., Cheryl A. Palmer, M.D., Jiri Vitek, M.D., Jui-Chang Tsai, M.D., Heidi L. Weiss, Ph.D., G. Yancey Gillespie, Ph.D.

Brain Tumor Research Laboratories, Division of Neurosurgery (CKG, SB, GYG), Division of Neuropathology (CAP), Division of Neuroradiology (JV), and Comprehensive Cancer Center Biostatistics Unit (HLW), University of Alabama School of Medicine, Birmingham, Alabama, and Department of Neurosurgery (J-CT), National University of Taiwan, Taipei, Taiwan, Republic of China

OBJECTIVE: Vascular permeability factor/vascular endothelial growth factor (VPF/VEGF), an endothelial cell-specific cytokine, induces proliferation of endothelial cells and increases vascular permeability dramatically. All gliomas secrete significant amounts of VEGF, whereas meningiomas are variable in expression. Thus, we sought to determine whether the extent of VPF/VEGF expression in meningiomas correlated with differences in brain edema associated with these tumors.

METHODS: Meningioma tissue samples from 37 patients (15 men, average age 65 ± 13 yr; 22 women, average age 60 ± 10 yr) who underwent surgery at or were referred to the University of Alabama Hospital were examined retrospectively for the extent of expression of immunoreactive VPF/VEGF. Additionally, peritumoral edema was assessed on a blinded basis radiographically from preoperative magnetic resonance imaging scans. Selected specimens were examined by in situ hybridization to document the source of VPF/VEGF.

RESULTS: The predominant meningioma subclassifications were transitional (57%) or meningothelial (27%) subtypes. VPF/VEGF immunoreactivity ranged from 0 to 3.5, with a median value of 2 on a subjective 5-point scale; magnetic resonance imaging-assessed edema ranged in extent from 0 to 4 (subjective 5-point scale), with a median value of 2.5. The correlation of determination (R^2) of magnetic resonance imaging-assessed tumor edema rating and VPF/VEGF staining intensity rating was 0.6087 ($r = 0.78$; $P = 0.0001$). In situ hybridization localized VPF/VEGF messenger ribonucleic acid in meningioma cells and not in normal parenchymal brain cells.

CONCLUSION: These data suggest that meningioma-associated edema may be a result of the capacity of meningioma cells to produce VPF/VEGF locally, leading to increased tumor neovascularization and enhanced vascular permeability. (Neurosurgery 40:1269–1277, 1997)

Key words: Angiogenesis, Edema, Meningioma, Vascular permeability, VEGF

A significant proportion of meningiomas are associated with peritumoral edema, which may result in alterations of normal brain function. The cause of meningioma edema is unclear. Factors thought to contribute to meningioma-associated edema have included meningioma-secreted cytokines and vascular obstruction (1–3, 5, 6, 9, 16, 20, 22, 30–32, 35, 36, 46–48). Neuroanatomists have demonstrated that the blood vessels of meningiomas do not have tight junctions that would enable them to limit fluid extravasation (45, 46). However, a tumor that simply compresses brain with an intact pial surface theoretically need not cause edema from extravasated fluid. Several authors have suggested that such tumors may be producing chemical mediator(s) that cause vascular proliferation in the tumor as well as breakdown of the blood-brain barrier in the underlying brain (34, 36, 46). Moreover, an indistinct relation between meningioma edema and neovascularity has been supported by several lines of evidence. Hemangiopericytic meningiomas, although infrequent, are associated with edema (9, 46). Additionally, meningiomas that derive a blood supply from both intrinsic

cerebral branches and meningeal branches are associated with an increased level of edema (21).

During the past 15 years, there has been an increasing amount of basic biochemical research examining the role of individual compounds that are associated with tumor edema. Nagy (33), Senger et al. (43, 44), Criscuolo (10), and Criscuolo et al. (11, 12) have been instrumental in establishing vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) as a causal agent in tumor-generated edema. As the bifunctional name suggests, VPF/VEGF is both an angiogenic factor and vascular permeability factor and may provide the molecular link between meningioma vascularity and peritumoral edema.

VPF/VEGF is a potent and specific endothelial cell mitogen in vitro (27) and induces angiogenesis in vivo. VPF/VEGF is 1000-fold more potent than histamine in inducing capillary permeability (8). Taken together, these important biological activities indicate that VPF/VEGF may play major roles in the angiogenesis and increased edema characteristic of certain meningiomas.

Recent studies have demonstrated the presence of VPF/VEGF messenger ribonucleic acid (mRNA) in meningioma tissues and cell lines (4, 41, 49). In this study, we examined the relation between VPF/VEGF protein immunoreactivity in meningiomas with peritumoral edema as assessed by magnetic resonance imaging (MRI) scan.

CLINICAL MATERIALS AND METHODS

Case materials

Paraffin blocks of surgical specimens were chosen from recent archival materials maintained by the Department of Pathology, Division of Anatomic Pathology based on two essential criteria, as follows: 1) a final histopathological diagnosis of meningioma, and 2) the availability of radiographic films from preoperative MRI studies performed at the University of Alabama in Birmingham or at outside clinical facilities. Routine hematoxylin and eosin-stained sections were freshly prepared to establish that the tissue sections to be studied were representative of the final diagnosis. Specimens that contained extensive artifact (because of electrocautery or autolysis) were excluded. Subclassification of meningioma by histological class was determined on the basis of the 1993 World Health Organization criteria (26) by one of the authors (CAP). Gender identification and age were obtained from the surgical pathology clinical history and later confirmed by blood bank records.

Blood grouping

Surgical specimens were selected on the basis of surgical pathology number, medical record number, and gender. This data was cross-referenced with blood bank record data of the University of Alabama in Birmingham to determine the blood type and Rh factor for each patient.

Edema rating

In most cases, because of the retrospective nature of this study, actual pixel data from scan tapes could not be accessed to allow direct quantitative comparisons between T1- and T2-weighted gadolinium images. Therefore, preoperative MRI scans were evaluated by an experienced neuroradiologist (JV) and rated with an arbitrary 5-point edema scale, as follows: absent = 0, minimal/equivocal = 1, mild = 2, moderate = 3, and intense = 4. Tumor edema was evaluated using both T2- and proton-weighted images. Guideline parameters for rating the severity of cerebral edema also included the following criteria: bihemispheric cerebral edema was rated as severe, presence of midline or ventricular shift was rated at least as moderate, and cases that exhibited definitive scattered areas of peritumoral enhancement with no brain shift were rated mild or moderate. Combined ratings (e.g., mild [value = 2] to moderate [value = 3] edema) were coded as the mean value of the two ratings (value = 2.5).

Anti-VPF/VEGF monoclonal antibody

Monoclonal antibody 5C3.F8 was produced in our laboratory by immunizing BALB/c mice with multiple sequential injections of purified human glioma-derived VPF/VEGF and a VPF/VEGF peptide containing amino acids 35 through 58. Hybridomas from several fusions were screened and one, designated 5C3.F8 (an immunoglobulin G_1), was chosen based on antigen recognition and avidity to recombinant human VPF/VEGF. Monoclonal antibody 5C3.F8 specifically recognized recombinant human VEGF/VPF resolved by 10% polyacrylamide gel in 1% sodium dodecyl sulfate (*Fig. 1, left lane*), as well as a similarly migrating molecule in a saline extract of meningioma tumor tissue (*Fig. 1, right lane*). Monoclonal antibody 5C3.F8 binding by Western blotting was visualized by sequential application of alkaline phosphatase conjugated goat anti-mouse immunoglobulin and 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium. Binding to

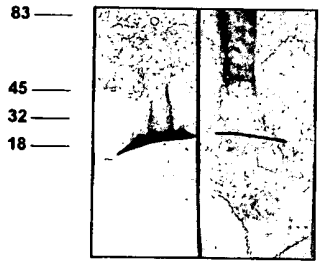

FIGURE 1. Mouse monoclonal antibody 5C3.F8 recognized human VEGF/VPF resolved by electrophoresis through 10% polyacrylamide gel in 1% sodium dodecyl sulfate. In the *left lane* (*rhVEGF*), a single band of recombinant human VEGF (1 μg) was detected with an estimated M_r of 26,000 through 28,000. In the *right lane*, a band with a similar electrophoretic mobility is observed in a saline extract obtained from freshly homogenized human meningioma tissue; approximately 70 μg of protein by Bradford assay was loaded. Prestained molecular weight markers (Bio-Rad Labs, Hercules, CA) were bovine serum albumin (*83 kD* [M_r, 83,000]), ovalbumin (*45 kD* [M_r, 45,000]), carbonic anhydrase (*32 kD* [M_r, 32,000]), and lysozyme (*18 kD* [M_r, 18,000]).

recombinant VEGF could be ablated by absorption of monoclonal antibody 5C3.F8 to meningioma tissues (data not shown). Monoclonal antibody 29.1.1 (Sigma Chemical Co., St. Louis, MO), an unrelated antibody directed against the epidermal growth factor receptor, was demonstrated to stain a significant number of meningiomas. For this reason, it was used as a positive control for immunohistochemical technique in selected cases, but was not included in the data presented. Incubation of tissue sections in 3% bovine serum albumin without primary antibody served as a negative control.

Tissue specimens and immunohistochemistry

All tissue samples selected had been formalin-fixed and paraffin-embedded. Sections 8 to 10 μm thick were deparaffinized, rehydrated in phosphate-buffered saline, and postfixed by immersion in sodium citrate with microwave antigen recovery. Representative slides were stained with hematoxylin and eosin for standard histological diagnosis. Postfixed sections were exposed to 3% bovine serum albumin alone or mouse monoclonal antibody (60 min at room temperature) and washed before incubating with biotinylated horse anti-mouse immunoglobulin. Avidin-biotin-horseradish peroxidase complex was added to washed sections to detect bound immunoglobulins according to the manufacturer's directions (Vector Labs, Burlingame, CA). Antigen-dependent color development was achieved by incubating reacted tissue samples with diaminobenzidine (10 μg/ml in 0.05 mol/L tris(hydroxymethyl)aminomethane, pH 7.2). Tissues were counterstained with Mayer's hematoxylin, dehydrated, and mounted in Eukitt medium for microscopic inspection and photomicrography.

Immunohistochemical grading

Peroxidase-stained hematoxylin-counterstained sections and controls were rated on an arbitrary 0 to 4 point scale based on staining intensity that was interpreted as relative VPF/VEGF immunoreactivity by two observers (CKG, SB) blinded to the study. Specimens with high background staining, high levels of artifactual staining, or extensive peroxidase "edge artifact" were repeated or eliminated from the series. Ratings were arbitrarily designated, as follows: no staining = 0, equivocal staining = 1, mild staining = 2, moderate staining = 3, and intense staining = 4. Staining intensity scores represent the mean of the values assigned by the observers. The location of the vascular immunoreactivity was determined on the basis of simplified histological criteria for vasculature. Vessels with calibers of arterioles, capillaries, and venules were classified as "small vessels," whereas other vessels were categorized as "large vessels." Immunostaining of blood vessels in the tissue section was graded as positive if there were three or more large unequivocally stained blood vessels per 400× field or more than 12 large plus small boldly stained blood vessels per 400× field. When examined microscopically, the tumor regions were classified as "outer tumor," which corresponded to tumor adjacent to brain or dura, and inner tumor that corresponded to tumor within the core of the meningioma.

In situ hybridization

In situ hybridization was performed on formalin-fixed paraffin-embedded tumor tissue sections. Full-length (685 base pairs) VPF/VEGF RNA probe for the 165 amino acid isoform (provided by Dr. Judith Abraham, Scios Nova, Mountain View, CA) was labeled with digoxigenin using Dig-11-UTP (Boehringer Mannheim, Inc., Indianapolis, IN) in a typical 50-μl transcription reaction. Tissue sections were deparaffinized and postfixed in 4% paraformaldehyde for 4 hours at room temperature. Sections were washed in phosphate-buffered saline and treated with proteinase K (Sigma; 1 μg/ml for 15 min at room temperature). Sections were washed with deionized H_2O twice. Deoxyribonuclease incubation treatment was administered at 37°C to degrade deoxyribonucleic acid present within the tissues. Sections were treated with levamisole (10 mg/ml, 37°C for 30 min) to inactivate endogenous alkaline phosphatases, washed with 2× standard saline citrate, and hybridized overnight with sense and antisense probes (50 ng) at 42°C in 1 ml of hybridization buffer (50% formamide, 4× standard saline citrate, 100 μg denatured salmon sperm deoxyribonucleic acid, 10% dextran sulfate). After extensive washes in phosphate-buffered saline, the hybridized RNA was detected on tissue sections using antidigoxigenin antibody conjugated with alkaline phosphatase (Boehringer-Mannheim). Sections were incubated with 5-bromo-4-chloro-3-indolyl phosphate/nitroblue tetrazolium substrate for 5 to 7 minutes and mounted in polyvinyl alcohol mounting medium.

Statistical analysis

The SAS System (SAS Institute, Cary, NC) available on an Alpha 3800S computer operated by the Biostatistics Unit of the Comprehensive Cancer Center was used to perform all statistical analyses. Spearman correlation coefficient was calculated to evaluate the magnitude of the linear association between edema rating and VEGF immunoreactivity rating. A weighted linear regression was used to model brain edema with VEGF expression. This enabled us to fit a linear regression model to our data and to account for the heteroscedasticity of the variance of the dependent variable of different values of the independent variables. The reciprocal of the variance of edema rating for each value of VEGF expression was used as the weight in the regression model. Students' t tests or Mann Whitney rank sum test were applied to determine gender differences, blood type differences, and histological differences with regard to edema, and VPF/VEGF immunoreactivity. χ^2 statistic was applied to analysis of blood types and gender differences as they related to vascular staining.

RESULTS

Patient population

Thirty-seven patients with meningioma according to clinical and histological criteria were included in this study (*Table 1*). Initially, tissue blocks for 63 patients were selected, but 26 of these had to be excluded based on incomplete medical, laboratory, or radiological information, or based on fixation

TABLE 1. Data Obtained for Each of the 37 Meningioma Cases[a]

Patient Number	Meningioma Histology[b]	MRI Edema Rating[c]	VEGF Immunostaining[c]	VEGF Immunostaining		Large Vessel Staining (VEGF)[d]	Large and Small Vessel Staining	Age (yr)/Sex	Blood Type[e]
				Outer	Center[c]				
1	Meningothelial	4	2	1	3	−	−	56/M	O+
2	Transitional	3.5	3.5	3.5	2	+	−	63/F	O+
3	Transitional	3.5	3	3.5	2	−	−	78/M	O+
4	Meningothelial	3.5	3.5	4	2	+	−	62/F	O+
5	Psammomatous	3	3.5	3.5	3	−	−	73/F	A+
6	Meningothelial	3	2	2.5	2	−	−	46/F	O+
7	Secretory	3	3	3	2	+	−	62/F	O−
8	Meningothelial	3	2.5	3	1.5	+	+	67/M	B+
9	Transitional	3	3.5	4	3.5	+	+	54/F	O+
10	Meningothelial	3	3	3	1	+	−	55/M	O+
11	Meningothelial	3	3	4	2	+	−	68/M	B+
12	Transitional	3	2	3	2	+	+	58/F	O+
13	Transitional	3	0.5	1	1	−	−	48/F	O+
14	Chordoid	2.5	2.5	3.5	2.5	−	−	85/M	O+
15	Transitional	2.5	3.5	3.5	3.5	+	−	79/M	O+
16	Transitional	2.5	2.5	3	2.5	+	+	51/M	O+
17	Transitional	2.5	3	2.5	1	+	−	60/F	O+
18	Transitional	2.5	1.5	2	1	+	+	58/F	O+
19	Psammomatous	2	3	3	2	−	−	65/F	B+
20	Fibroblastic	2	1	1.5	0.5	−	−	58/F	AB−
21	Meningothelial	2	2.5	2.5	1.5	±	−	73/M	A+
22	Transitional	2	2.5	2.5	2.5	+	+	79/M	B+
23	Transitional	1.5	1.5	2	1	−	−	48/F	O+
24	Transitional	1.5	2.5	4	2.5	+	−	66/M	A+
25	Transitional	1.5	0.5	0.5	0.5	+	−	33/F	A+
26	Meningothelial	1.5	2.5	3.5	2.5	+	+	59/M	O+
27	Transitional	1	3.5	3.5	3.5	−	−	69/F	O+
28	Meningothelial	1	1.5	1.5	1.5	+	−	67/F	O−
29	Transitional	0.5	0	0	0	−	−	76/F	A+
30	Transitional	0.5	0.5	0.5	0.5	−	−	76/F	O+
31	Transitional	0.5	0.5	0.5	0.5	−	−	58/F	O+
32	Meningothelial	0.5	2	3	2	+	−	65/M	
33	Transitional	0.5	1	1	1	−	−	59/F	O+
34	Transitional	0	2	1.5	2.5	−	−	35/M	O+
35	Fibroblastic	0	0	0	0	−	−	64/M	
36	Transitional	0	1	0	1	−	−	68/F	A+
37	Transitional	0	3	4	2.5	+	+	58/F	A−

[a] MRI, magnetic resonance imaging; VEGF, vascular endothelial growth factor.

[b] Subclassification of meningioma by histological class was made on the basis of the 1993 World Health Organization criteria.

[c] MRI edema rating and VEGF immunostaining intensity were rated on a 0–4 scale or grading criteria: 0 = negative; 1 = minimal, 2 = mild; 3 = moderate; and 4 = intense. The ratings from two observers for each case were averaged, which frequently resulted in half values. For example, the relative edema for Patient 2 is encoded as 3.5 and represents moderate to intense edema.

[d] VEGF immunostaining of the vasculature was recorded as present (+) if three or more large unequivocally stained blood vessels per 400× field or more than 12 large plus small boldly stained blood vessels per 400× field were seen. (−) indicates that immunostaining was absent or not significant. A single case (Patient 21) was ranked as equivocal (±). Small vessels were classified as venules, capillaries, or arterioles.

[e] Gender identification and age were obtained from the surgical pathology clinical history and later confirmed by blood bank records used to determine blood type and Rh factor for each patient.

artifact, high normal tissue component, or irreproducible antibody staining patterns.

Case overview

The final study group of 37 cases (*Table 1*) was composed of 22 women (59.5%) with ages ranging from 33 to 76 years (mean, 60 yr) and 15 men (40.5%) with ages ranging from 35 to 85 years (mean, 65 yr). Twenty-one (56.7%) cases were histologically subclassified as transitional meningiomas, and the remainder of cases were classified as either meningothelial (27%), fibroblastic (5.4%), psammomatous (5.4%), chordoid (2.7%), or secretory (2.7%). Overall, VPF/VEGF immunoreactivity grading (*Table 1*) in these tumor sections ranged from 0 to 3.5 (median = 2). Radiographically, tumor edema rating

ranged from 0 to 4 (median = 2.5). Of the 35 patients for which blood type data were available, 23 (65.7%) had blood Type O, 7 (20%) had blood Type A, 4 (11.4%) had blood Type B, and 1 (2.9%) had blood Type AB.

VPF/VEGF immunoreactivity

There was a great deal of variability for VPF/VEGF immunoreactivity patterns among the 37 tumors examined (*Fig. 2*). Some tumors exhibited homogeneous staining from cell to cell, whereas others seemed to contain intensely staining cells next to nests of cells that were without staining. Ratings of overall staining intensity were based on the intensity of the majority of cells within the tumor tissue. Tumors were further graded based on staining in the "outer" or "center" portions, corresponding to areas adjacent to dura or deep within the tumor bed, respectively (*Table 1*).

In situ hybridization

A total of six cases were randomly chosen for VPF/VEGF in situ hybridization in an attempt to assess the extent to which tumor cells or nontumor cells possessed mRNA transcripts for VPF/VEGF and were synthesizing the molecule directly. All samples demonstrated variable degrees of antisense hybridization of the VPF/VEGF probe (*Fig. 3A*). In contrast, the sense probe revealed no demonstrable hybridization (*Fig. 3B*). Normal dura demonstrated no significant VPF/VEGF hybridization, even in areas that were adjacent to intensely hybridization positive meningioma cells (*Fig. 3C*). No attempt was made to correlate VPF/VEGF probe antisense hybridization to either peritumoral edema or VPF/VEGF immunoreactivity.

Relation of VPF/VEGF expression and edema

There was a statistically significant positive correlation between the ratings of tumor edema and VPF/VEGF overall intensity (*Fig. 4*, $r = 0.78$, $P = 0.0001$). Tumor edema also correlated with outer tumor VPF/VEGF immunoreactivity intensity ($r = 0.44927$, $P = 0.055$) and correlated less well with center VPF/VEGF immunoreactivity intensity ($r = 0.3061$; $P = 0.065$).

Histological characteristics

Meningiomas subclassified as meningothelial generally displayed slightly higher degrees of peritumoral edema than transitional meningiomas (Mann Whitney rank sum test, $P = 0.013$). Similarly, meningothelial meningiomas demonstrated higher degrees of VPF/VEGF immunoreactivity than transitional meningiomas (Student's t test: $t = 1.48$, $df = 35$; $P = 0.14$); however, this relation was not statistically significant. There was no obvious relation between large or small blood vessel staining and any of the other variables examined.

Patient population characteristics

Although the majority of patients were women, men and women demonstrated similar degrees of tumor edema (Student's t test: $t = 1.42$, $df = 36$; $P = 0.16$). There was no difference between men and women with regard to either tumor histological subtype or blood type. We originally chose to examine blood types in this study because our preliminary findings (CK Goldman, J-C Tsai, GY Gillespie, unpublished data) suggested a possible relation between blood type and endothelial staining. This phenomenon, however, was not repro-

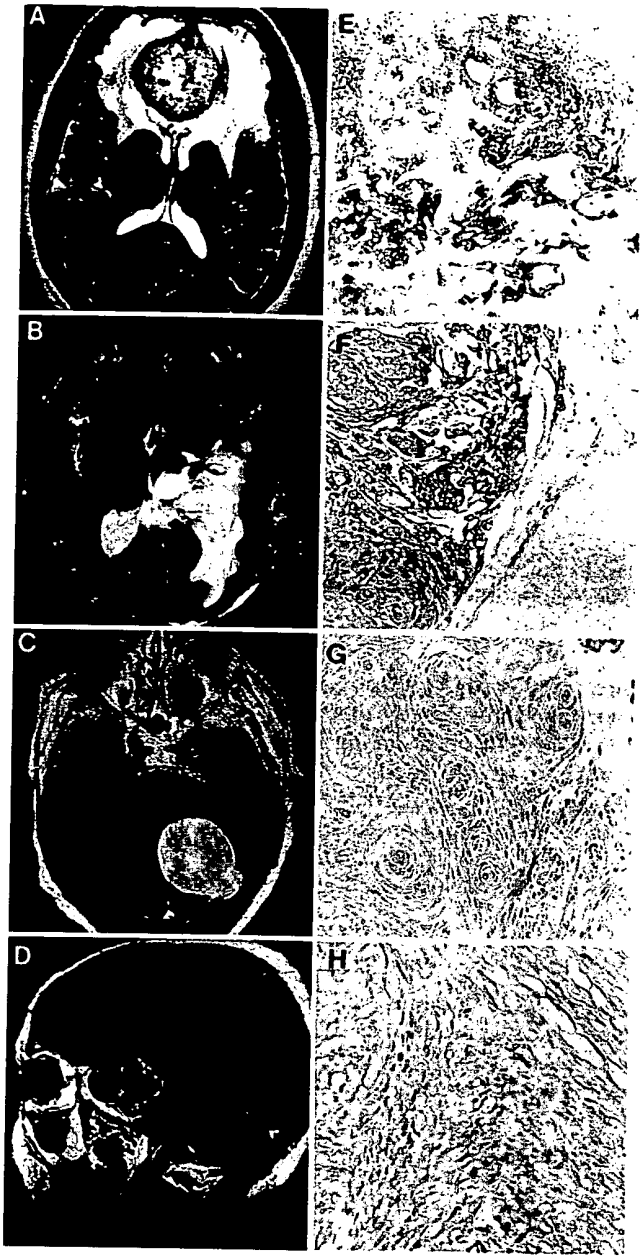

FIGURE 2. Representative preoperative MRI images (*left panels*) with the corresponding tumor tissue resected from the same patient (*right panels*) and tested for immunoreactivity for VEGF/VPF. These selected images demonstrate the range of edematous changes estimated from T2- or T1-weighted MRI scans (*Panels A–D*), as well as the differences in intensity and distribution of monoclonal antibody 5C3.F8 binding (*Panels E* and *F*).

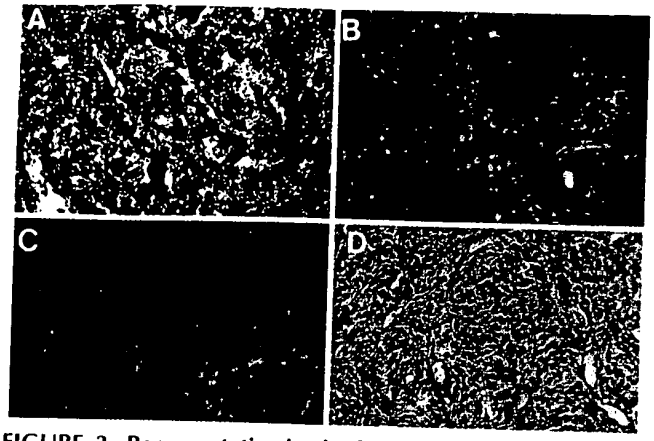

FIGURE 3. Representative in situ hybridization results using digoxigenin-labeled antisense (*Panels A* and *C*) or sense (*Panel B*) probes detected with enzyme-linked antidigoxigenin antibodies. Hematoxylin and eosin-stained section adjacent to those used in *A* and *B* (*Panel D*). In *Panel C*, meningioma cells invading dura (*darkly stained areas*) demonstrated strong hybridization with VEGF/VPF antisense probe.

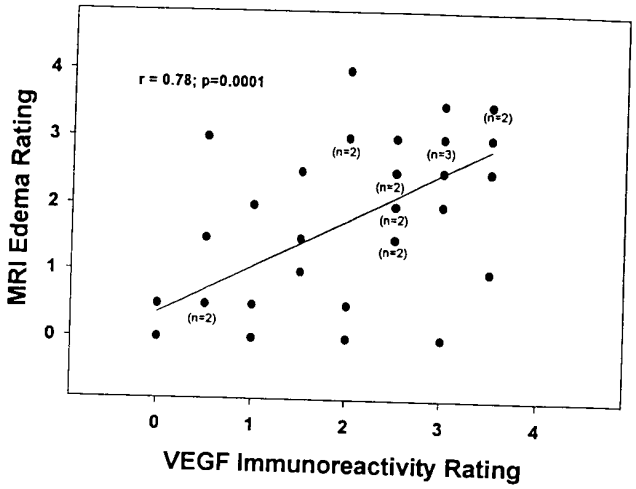

FIGURE 4. A positive correlation between overall VEGF immunoreactivity staining of resected meningioma tissues and tumor edema rating based on preoperative MRI scans was statistically significant ($r = 0.78$, $P = 0.0001$). The number of cases with identical ratings are indicated by *(n = x)* below the data points.

duced in this study. There was no obvious relation between blood type and any of the other variables tested. Unexpectedly, the majority of patients in this study had blood Type O. When compared with the distribution of blood types from blood donors in a typical population (28), blood Type O (normal population = 30%) is disproportionately overrepresented in this study (65%), whereas blood Type A (normal population = 50%) was underrepresented in this study population (20%).

DISCUSSION

VPF/VEGF is a potent endothelial cell-selective glycoprotein that is secreted in at least three, and possibly four, soluble or membrane-associated isoforms by many tumor types and several kinds of normal tissues (4, 8, 11, 18, 27, 33, 37, 43, 44). VPF/VEGF has been observed in tumors and embryonic tissues, suggesting a possible role in the establishment of blood supply in rapidly dividing cells. Although some normal tissues produce VPF/VEGF mRNA, tumor cells in comparison markedly overexpress VPF/VEGF mRNA and protein (4, 43, 44). An important role for VPF/VEGF in tumor pathophysiology is supported by the finding that blocking VPF/VEGF antibodies eliminate growth of human tumor xenografts in mice with severe combined immunodeficiency (25). By itself, VPF/VEGF has been observed to induce angiogenesis and increased vascular permeability in vivo, and therefore, may play a central role in neovascularization and tumor stroma generation (44). VPF/VEGF mRNA has been documented in meningioma cell lines, and its levels have been observed to correlate with the extent of meningioma vascularity.

Our data demonstrate a direct correlation between VPF/VEGF immunoreactivity and peritumoral enhancement and thereby support a role for VPF/VEGF in meningioma edema pathophysiology. Additionally, our results confirm the presence of VPF/VEGF mRNA in meningiomas by in situ hybridization. The finding that VPF/VEGF immunoreactivity correlates with meningioma peritumoral edema supports its role in meningioma pathophysiology and supplements the finding that VPF/VEGF mRNA expression in meningiomas correlates with the development of new blood vessels (41). From a prognostic perspective, malignant meningiomas are generally associated with clinically important edema, whereas the absence of edema indicates a meningioma that will have a more benign course (19, 29, 38). Furthermore, the more extensive the tumor-associated edema, the greater the likelihood that clinically significant symptoms will occur earlier and may require surgical intervention. For this reason, although the presence of edema may indicate a more aggressive tumor, any biological analysis of the role of VPF/VEGF in meningioma tumor biology based on retrospective tissue sampling will be potentially confounded by earlier surgical removal of the tumors that generate more edema. Because of this selection bias, our study population may have higher VPF/VEGF protein expression than is present in the population of all patients with meningiomas.

The mechanism of tumor edema, although speculative, has been attributed to a combined effect of mechanical forces, e.g., venous or cerebrospinal fluid obstruction and soluble secretory factors (1–3, 5, 6, 9, 16, 20, 22, 30–32, 35, 36, 46–48). Specific correlates of tumor size (2, 7, 46), prostaglandin production (9), tumor cellularity (46), histological type (16, 36), and the presence of progesterone receptors (3, 6, 17, 31, 32, 39) have been postulated to be determinants of peritumoral edema. From a cellular perspective, there may be a meaningful relation between VPF/VEGF, progesterone receptors, and prostaglandin levels. In rodent endometrium, the expression of VPF/VEGF is up-regulated by treatment with progesterone in the presence of estrogen (13). Although our preliminary studies examining the effect of progesterone and estrogen in a

single meningioma cell line (CH157 MN) have been inconclusive (data not shown), circumstantial clinical evidence would suggest that female hormone receptor stimulation might increase VPF/VEGF expression in meningiomas. Clinical data linking fluctuations in the levels of female sex hormones to meningioma pathophysiology, possibly attributable to VPF/VEGF, include the findings that meningiomas frequently become symptomatic during pregnancy and that meningioma-associated symptoms sometimes fluctuate with the menstrual cycle (14, 17, 39).

Glucocorticoids mitigate the effects of peritumoral edema on patient symptomatology. Although the effects of glucocorticoids on VEGF secretion is unclear, dexamethasone does inhibit the in vitro correlate of vascular permeability, namely endothelial cell calcium transients. The ability of dexamethasone to block VEGF-induced calcium transients in endothelial cells suggests that dexamethasone suppresses vascular permeability by interfering with the VEGF-induced signals within the endothelial cell. Unfortunately, data relating peritumoral edema with the administration of glucocorticoids during the time of the MRI scan or during surgery are not available for the patients in this study. Without this data, we suggest that our study would tend to underestimate the relation between brain edema and VEGF/VPF immunoreactivity.

Previous studies examining meningioma peritumoral edema have used imaging studies such as computed tomographic or MRI scans as an indicator of peritumoral edema (7, 15, 23, 24, 40, 42). There is, however, a paucity of data demonstrating that the peritumoral magnetic resonance changes are coincident with actual brain edema. One study that has examined this observed that peritumoral magnetic resonance changes usually ascribed to edema actually represent an increased capillary density (7). The authors, however, did not exclude the possibility that the increased capillary density may have been accompanied by edema. Because of the dual angiogenic and vascular permeability properties of VPF/VEGF, it would not be unexpected to find both increased capillary density *and* increased vascular permeability. Nevertheless, the assumption that enhanced T2-weighted signal on MRI, or hypodensity on computed tomographic scan, represents peritumoral edema will remain unproved until actual cytological changes in brain tissues that surround tumors are better defined. As this correlative information becomes available, it might be possible to obtain a quantitative measure of peritumoral edema by subtracting the T1-weighted gadolinium volume from that revealed by the T2-weighted hyperintense signal.

Our initial interest in examining blood grouping was related to our finding that some meningiomas had densely staining capillaries for VEGF, whereas other tumors were completely devoid. This was reminiscent of staining patterns observed with some antibodies that cross-react with certain blood types. However, we did not find a relation between blood types and either VEGF staining or vasogenic edema. Interestingly, our population of meningioma patients had an overrepresentation of blood Type O patients. It was unclear whether this is a bona fide relation between blood type and predisposition for development of meningiomas or whether this finding represents a chance occurrence because of sampling bias. Larger sample sizes might need to be examined to establish this possible relation, if any.

In summary, our finding that VPF/VEGF immunoreactivity has a significantly positive correlation with meningioma peritumoral edema indicates a causal biological relation. However, it does not exclude other factors that may also be important.

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COMMENTS

This study implicates the role of vascular permeability factor/vascular endothelial growth factor (VPF/VEGF) as both angiogenic and vascular and responsible for inducing edema formation associated with meningeal tumors. Although other studies have noted increased message levels of VPF/VEGF with meningioma samples and primary cell cultures, an association between VPF/VEGF immunoreactivity and edema patterns on diagnostic imaging studies has been lacking until this current investigation. The authors also strengthened their immunoreactivity data with in situ hybridization to confirm mRNA expression in these meningiomas. It is also interesting to speculate, although little data is provided to support this, whether certain sex steroid hormones may actually up-regulate VPF/VEGF expression and thereby account for the exacerbation of symptoms and signs that may occur in women during pregnancy and menstrual cycles. Further work is needed to verify this claim.

In reviewing further series of correlative imaging studies from this group, it will be interesting to note whether the size of the tumor, as well as the size of the peritumoral edema, correlates significantly with the quantitative expression of VPF/VEGF. This would make the investigation of an antibody or antisense oligonucleotide against VPF/VEGF even more logical and appropriate.

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This work follows previous reports by Gillespie and colleagues in which the role of VEGF was examined in human malignant gliomas. In the present report, the authors have observed that brain edema is significantly associated with increased VEGF expression by immunohistochemistry in human meningiomas. Brain edema was assessed using an arbitrary 5-point grading scale applied to preoperative magnetic resonance imaging scans. There was no correlation between histological subtype of meningioma and VEGF expression nor was there a relationship observed between VEGF expression and the presence of large or small vessels within the tumors. However, none of the tumors examined were so-called angioblastic meningiomas, which would have been an interesting subcategory of meningioma to examine for VEGF expression. All neurosurgeons have had experience with small meningiomas that are associated with massive cerebral edema. Goldman et al. provide additional evidence that this unusual phenomenon may be ascribed at least in part to VEGF expression in human meningiomas.

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This article provides some interesting observations on the biochemical aspects of the cause of brain edema in meningiomas. The authors examined the significance of VPF/VEGF as a causal agent in meningioma-generated brain edema. The results demonstrate a moderate positive correlation between the edema intensity score and VPF/VEGF immunoreactivity. Because VPF/VEGF is both angiogenic and vascular, it is suggested that meningioma cells produce VPF/VEGF, leading to increased neovascularization and enhanced vascular permeability. Increased vascular permeability is the main source of edema fluid extravasation.

During the past 5 years, an increasing amount of data has emerged demonstrating that human malignant glioma cells secrete biochemical factors that increase capillary permeability and that glucosteroids may inhibit the effect of these factors. Thus the identification of such capillary permeability factors in meningiomas is an important step to better understand the process of edema formation.

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The importance of VPF, also known as VEGF, in either peritumoral edema formation and/or neovascularization has been demonstrated in a wide variety of the central nervous system neoplasm. The present study adds meningiomas to this group, offering convincing evidence that VPF/VEGF is involved in peritumoral edema formation. The authors demonstrate that the intensity of VPF/VEGF immunoreactivity in meningiomas correlates with the rating of tumoral edema as determined on a relative scale (0–4) from preoperative magnetic resonance imaging scans. This correlation, however, was not always perfect; there was no strong association with outer tumor VPF immunoreactivity, and even less association with VPF immunoreactivity in the center of these tumors. The varying degree of the association may reflect the involvement of other factors that have been observed by other groups to be implicated in the formation of peritumoral edema in meningiomas. The list includes eicosanoids, platelet-aggregating factor, cytokines, etc., which were not investigated in the present series. A study that would examine simultaneously the expression of several of these factors in meningiomas, as well as their correlation with edema formation, would be informative. Nevertheless, the demonstration of VEGF mRNA in meningiomas strongly indicates the possibility of meningeal production of this peptide and its pathophysiological role. Although recent experimental studies have demonstrated that antisense VEGF gene therapy may result in the inhibition of tumor (glioma) growth, the concept that a similar therapy could be used to prevent the recurrence of meningiomas after surgery deserves thought.

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